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ON THE

PHAGOCYTES

OF THE

ALIMENTARY CANAL.

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BY

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On the Phagocytes of the Alimentary Canal.

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With Plate XXXI.

During the last few years our knowledge of the pathological appearances found in animals suffering from an infectious disease has, thanks to M. Metschnikoff's labours, been considerably extended. Infectious diseases for some years past were known to be caused by the presence of pathogenic micro-organisms in the tissues of animals—each malady being due to a distinct specific germ—and most of these specific germs had been isolated and cultivated (e. g. typhoid fever, tuberculosis, pneumonia, diphtheria, &c.). Some of these organisms moreover, when inoculated into animals, gave rise to morbid symptoms and to pathological appearances similar to those seen when man is invaded by them; whilst others (e. g. typhoid bacillus) proved fatal to some species of animals only (e. g. mice), though the clinical aspect and post-mortem lesions when the typhoid bacilli were injected in mice did not in any way resemble those produced by the same bacilli when the human species was attacked by them.

It was, however, soon noticed that some species of animals were able to resist the inoculation of even large numbers of some kinds of specific germs. Animals belonging to another species, on the other hand, suffered but little when few specific germs were injected into their system, but did when a larger number of the same germs was introduced. Clinical observations had already demonstrated the fact that when an animal recovered from an infectious disease that animal was unable to contract it again. It was said to be vaccinated against that particular kind of micro-organisms. Actual experiment soon showed that it was possible to artificially vaccinate an animal against some fungi by the inoculation of an attenuated form of the same germs (Pasteur, Chauveau), or even by the injection of the poisons secreted by the same micro-organisms in artificial cultures (Charrin, Salmon and Smith, Roux and Chamberland, Chantemesse and Widal). The bacillus of anthrax, for instance, having no or very little harmful influence on a dog, on a vaccinated sheep, rabbit, or guinea-pig, was always mortal to the three latter species of animals if they were not previously subjected to a preventive inoculation of the attenuated anthrax bacilli. The Bacillus pyocyaneus injected into the rabbit's veins proved fatal to that animal in a period varying from twenty-four to forty-eight hours, but produced a temporary illness only when introduced into a rabbit vaccinated with 15 or 20 c.c. of the sterilised culture of the same bacillus.

Now, Metschnikoff[1] and Hess[2] have proved that the blood or lymph of the dog, of the vaccinated rabbit or guinea-pig, were most excellent media for the cultivation of the anthrax bacillus; in other words, the blood of these animals did not contain any chemical compounds capable of arresting the growth of that micro-organism. Another hypothesis assuming that micro-organisms secreted a vaccinating substance which, when introduced into the animal, remained stored up in its cells, thus protecting the latter against the attacks of the former, has been proved to be erroneous by the experiments of Professor Bouchard,[3] of Dr. Charrin, and myself.[4] These experiments showed that the vaccinating substances secreted by the Bacillus pyocyaneus in the rabbit's organism were excreted by the kidneys almost as soon as they were formed. Moreover, Dr. Charrin and myself[1] have seen that the sterilised blood of a rabbit dying after the inoculation of the Bacillus pyocyaneus often contained enough vaccinating substance to vaccinate another rabbit, provided the sterilised extract of blood was injected into the second animal twenty-four hours before the Bacillus pyocyaneus was introduced. The first rabbit, though saturated with vaccinating substances, was unable to resist the attacks of the Bacillus pyocyaneus.

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[1] Metschnikoff, 'Virchow's Archiv,' cxiv, Heft 3; 'Ann. de l'Institut Pasteur,' 1887.

[2] 'Virchow's Arch.,' Bd. cix.

[3] Bouchard, 'Compt. Rend. de l'Acad. des Sciences,' 4th June, 1888.

[4] Charrin and Armand Ruffer, ibid., 15th October, 1888.

The animal organism must not be compared to a cultivating medium which, after a particular kind of micro-organisms has grown in it for some time, contains substances having a harmful action on the micro-organisms producing them. The cells of an animal react against parasites; the kidneys excrete the poisons secreted by fungi in the diseased animal, for if the vaccinating substances are thrown out of the animal body the poisons producing paralysis, fever, &c., have the same fate.[2] A living being is therefore provided with various means of defence against the invasion of microbes which are always threatening it.

M. Metschnikoff's investigations have undoubtedly thrown a new light on this question.[3] He has shown that when pathogenic bacilli are inoculated into a vaccinated animal, or into an animal belonging to a species which successfully resists the attack of that particular kind of bacilli, some of the cells of these animals—cells to which he has given the name of macrophages and microphages—fight against their hosts, swallow them up, destroy, and digest them. The observer's eye on the microscope is able to follow the struggle for life in all its phases.

M. Metschnikoff has also demonstrated the fact that, if an animal be not vaccinated or does not normally possess a certain amount of resistance to pathogenic micro-organisms, its cells do not try to destroy these fungi, or, if they do make any attempts, they only absorb and digest very few of them. The parasites then multiply and kill the animal, partly by producing mechanical lesions, but mostly by poisoning it with the toxic substances they secrete. Vaccination against a contagious disorder, therefore, confers on these macrophages and microphages the power of destroying the specific organisms causing the disease.

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[1] Charrin and Armand Ruffer, 'Compt. Rend. Soc. d. Biologie,' March, 1889.

[2] Bouchard, loc. cit.; Charrin and Armand Ruffer, 'Comp. Rend. Soc. d. Biologie,' 1889.

[3] Metschnikoff. The best account of M. Metschnikoff's early researches will be found in 'Ann. de l'Institut Pasteur,' No. 7, 1887.

Now, in the alimentary tract various pathogenic organisms are frequently present. Indeed, some of them are constantly found there, even in healthy animals. There are few adult men who, at some time or other, have not swallowed the bacilli of typhoid fever or tuberculosis, though most have experienced no evil effects of them. The bacilli of diphtheria and pneumonia have been met with in the mouths of healthy subjects, who had never contracted these diseases. The septic vibrio of Pasteur is always present in the intestine of the rabbit, though unable to get through the walls of the gut. How is it, then, that the micro-organisms do not pass into the blood or lymphatics?

In order to throw light on this question it was necessary to see what takes place in the healthy animal, and to observe whether the numerous micro-organisms, normally present in the alimentary tract, make any efforts to force their way through the walls. It is well known that many of the infectious processes affecting the intestinal tract originate in the lymphoid tissues, namely, in the tonsils and Peyer's patches, and accordingly the first investigations concerning the penetration of micro-organisms through the intestinal walls have been made on the Peyer's patches (Ribbert and Bizzozero).

In this paper I shall only speak of the phagocytic processes taking place in the lymphoid tissues of the alimentary tract of healthy animals, pointing out afterwards how these facts throw some light on pathological observations. I must reserve for another opportunity an account of investigations as to the fate of pathogenic organisms artificially introduced into the alimentary tract, or as to that of fungi normally found in the intestinal canal when its walls are in any way wounded (strangulated hernia, &c).

I must be allowed here to thank M. Metschnikoff, under whose direction these investigations have been carried out at the Pasteur Institute in Paris. M. Metschnikoff has not only examined most of my slides, but he, with the greatest kindness, has placed his vast technical and literary knowledge daily at my disposal. To M. Pasteur my best thanks are due for kindly allowing me to work during many months in the institute which has been raised to him during his lifetime as a token of respect by a grateful humanity.

II.

The tonsils and Peyer's patches of animals are described as consisting of reticulated connective tissue and lymphoid follicles. Each may be considered as a lymphatic gland covered by a mucous membrane, and hollowed out by crypts lined by the same mucous membrane.

The mucous membrane of the inner aspect of the tonsil is covered by stratified epithelium. Below that epithelium the tissue is formed by reticulated connective tissue and closed lymphoid follicles. The cavities of the crypts contain epithelial cells which have been shed, leucocytes and micro-organisms.

The lymphoid follicles are joined together by a reticulated tissue containing small round cells. The reticulum as well as the spaces contained within its meshes, the size of the cells contained in them, may be very variable. The round-cells in the meshes of the lymphoid tissue divide, as Drews[1] has shown, by karyokinesis. I have also frequently been able to see these figures in my preparations. Stöhr[2] has proved that leucocytes always wander to the surface of the tonsil and fall into the cavity of the mouth.

The Peyer's glands are larger or smaller groups of lymph-follicles more or less fused with one another, and situated with their main part in the submucosa, but extending with their summit to the epithelium of the free surface of the mucosa (Klein). They are covered by a single layer of columnar epithelium, which is invaded by, and more or less replaced by, the lymph corpuscles of the adenoid tissue of the follicles (Stöhr).[1]

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[1] Drews, 'Arch. f. mikros. Anat.,' vol. xxiv, 1885.

[2] Stöhr, 'Arch. f. path. An. und Phys.,' xcviii.

Ribbert,[2] in 1885, noticed that by staining sections of the vermiform appendix by Gram's method numerous micro-organisms could be demonstrated in their interior. He further made the remark that many of these micro-organisms stained badly and showed signs of degeneration. He attributed this change to the action of the leucocytes of the Peyer's patches. Bizzozero[3] confirmed Ribbert's researches in the same year, but thought that the cells containing micro-organisms were not ordinary leucocytes, as they appeared to be larger.

Metschnikoff,[4] in 1877, examining the mucous covering of the surface of the tonsils, found in it numerous leucocytes crammed with micro-organisms.

The tissues which I used for my investigations were always prepared in the following way. The animals (rabbits, dogs, guinea-pigs) had always been kept in the Institute for some days beforehand, and appeared to be perfectly healthy. None of them had ever been used before for any experiments whatever. They were chloroformed to death, and as soon as they had ceased to breathe the tissues to be investigated were cut out and at once plunged into absolute alcohol, in which they were kept till properly hardened.

After being soaked in chloroform, ether, and paraffin they were cut after long (forty-eight hours) immersion in the latter. I usually at once glued them to the cover-glass with a mixture of glycerine and albumin, removed the paraffin with benzine, and after washing them for five minutes in absolute alcohol, and for an equal period in water, stained them with alum-carmine and gentian-violet, or by some other method (fuchsine, Löffler's blue).

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[1] Stöhr, see Heidenhain, 'Pflüger's Arch.,' 1888.

[2] Ribbert, 'Centrbl. f. Bakt.,' 1885.

[3] Bizzozero, 'D. med. Wochenschr.,' 1885.

[4] Metschnikoff, 'Ann. de l'Institut Pasteur,' No. 7, 1887.

If the free epithelial border of a rabbit's Peyer's patch be examined with a high power (Zeiss, oc. 3, obj. imm. $\frac{1}{18}$) it is noticed to have been invaded in many places by numerous leucocytes. I shall call these leucocytes microphages, on account of the power they undoubtedly possess of taking micro-organisms into their interior, and of destroying them. Stöhr[1] had noticed that these microphages (leucocytes) come up to the free surface throughout the whole length of the alimentary canal. They are, however, present in greater numbers between the epithelial cells covering the Peyer's patches than in other parts of the intestine. This is easily demonstrated by causing the section to pass through the lymphoid tissues and at the same time through the part of the intestine surrounding the patch; the microphages being more than twice as numerous between the epithelial cells covering the lymphoid tissues than between those placed over the neighbouring parts of the gut.

The microphages are sometimes more crowded in the epithelial lining at the top of the Peyer's patch; at other places, however, many more have emigrated into the epithelial border lining the crypts. These microphages are not only present near the attached ends of the epithelial cells, but they find their way, as Heidenhain[2] was the first to observe, even up to so high a level as the free border of the epithelium, and may fall into the intestinal cavity. I have been able to convince myself that these views were correct, by making numerous microscopical specimens of a recently killed guinea-pig or rabbit's intestinal contents, in which microphages are almost always found.

So numerous are the microphages between the epithelial cells that, at first sight, they sometimes appear to have destroyed and taken the places of all the epithelial cells.

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[1] Stöhr, loc. cit.

[2] Heidenhain, 'Pflüger's Arch.,' Supplem. Heft, 1888; 'Beitr. zur Histologie und Phys. der Dünndarmschleimhaut.'

On closer examination, however, it is plain that the latter have been merely pushed out of the way by the advance of the microphages, and crushed by them into the most extraordinary positions. It is almost always possible to make sure of the presence of the epithelial cells by looking for their nuclei, which differ greatly in shape and colouration from those of the invading microphages. Their nuclei, instead of being vertical as they ought to be, are often placed obliquely or even horizontally. The striated border of the epithelial lining, showing the cells composing it to be present, is also intact as a rule in all places, except where the leucocytes are actually about to fall into the intestinal cavity.

The nucleus or nuclei (for many are polynucleated) of these microphages are small, and stain deep red with alum-carmine. They are sometimes provided with one or two nucleoli which stain more deeply. The intranuclear reticulum is but poorly marked and often almost absent. I cannot say for certain whether the other large cells which will, under the name of macrophages, be described later on, do or do not penetrate into the epithelial lining of the rabbit's or dog's lymphoid patches, as I have never been able to demonstrate their presence there with absolute certainty. The guinea-pig's macrophages undoubtedly come right up to the free surface.

The interest of the section increases if the tissue be first stained with alum-carmine in the usual way, and secondly with gentian-violet according to Gram's method as modified by Weigert. The numerous micro-organisms, varying in shape, size, and number which line the intestinal cavity, assume a deep violet-blue colour, whilst the nuclei are stained red and the protoplasm of the cells of a pale rose colour. The layer of microbes does not as a rule actually touch the epithelial lining, but is separated from it by a small clear space. It would be almost impossible to describe the innumerable forms and sizes of these micro-organisms, but special attention must be called to the fact that all the fungi present in the cavity of the intestine, if they retain the colouring matter at all, are stained deep violet-blue. Some are, of course, of a slightly darker hue than others, but one never sees any degenerated organisms such as will be described later on; these forms of degeneration being only found in microbes which have been taken into the interior of living micro- or macrophages.

The leucocytes found between the epithelial cells occasionally contain numerous microbes and are therefore true microphages. There can be no doubt that the micro-organisms really lie in the interior of these cells, and not simply above or below them. As a rule they do not show any signs of degeneration as long as the microphages are still placed between the epithelial cells. Most of the microbes present in the cells are short, though some of them are of very large size (see figs. 8, 8 *bis*, 9). On the other hand, micro-organisms are never found in the epithelial cells except occasionally in one which had undergone mucoid degeneration. They are not found lying free between the epithelial cells, for, when there, they are always enclosed in a leucocyte. On superficial examination the epithelial cells sometimes appeared to contain microbes, but a closer scrutiny showed that these were always at a higher or lower level than the latter, and had been carried into that position by the knife or by some other artificial means. Of course, every leucocyte found in the epithelial border does not always contain microbes, but it is only now and then that such a one is met with.

Let it be stated here that these facts may be observed in all the Peyer's patches of rabbits wherever these are present in the intestinal canal, but the formation of macrophages and the destruction of microbes take place far more actively in some of them. The patch placed near the rabbit's ilio-cæcal valve and the vermiform appendix are the structures in which these processes are most marked. Whereas in the lymphoid patches of the small intestine six to twelve bacilli only may be present in one field of the microscope (oc. 3, obj. $\frac{1}{12}$ Vérick), I have counted more than two hundred micro-organisms in a single field of the deeper layers of the vermiform appendix.

If the tissue immediately below the epithelial border be now examined other characteristic cells attract the observer's attention. In sections carefully and deeply stained with alum-carmine or hæmatoxylin large mononucleated cells are seen lying scattered between the lymphocytes.

The drawings (figs. 1 to 15) give an exact representation of these cells, after the section has been stained with alum-carmine and gentian-violet. The size of one of these structures when fully developed is enormous if it be compared to that of the lymphocytes surrounding them; and, taking into account their size and their functions, they must be included under the class of cells to which M. Metschnikoff has given the name of macrophages.

The nucleus of a macrophage is always single, varies in size according to that of the cell, becoming larger as the latter increases, and may be twice or three times the size of that of an ordinary lymphocyte (see figs. 1—7, also fig. 10). It is sometimes round, more frequently oval, or even of a horseshoe shape. The different forms may possibly be due to their being cut across obliquely by the knife. The nucleus has often two or three nucleoli together with a very fine and delicate reticulum staining well with carmine or hæmatoxylin. The protoplasm of the macrophage is quite homogeneous, of a pale rose colour after the action of alum-carmine, or of a pale violet hue after being stained with hæmatoxylin. The protoplasm often contains large vacuoles (see figs. 15, 18, 19). The contours of the whole cell are sometimes quite round; sometimes, however, irregular (see figs. 7, 8 *bis*, 12). The occasional irregularity of shape is an argument in favour of the supposition that these cells are contractile, and therefore probably mobile, having been fixed by the alcohol at a time when, being on the march, they had altered their shapes. Other facts to be enumerated later on confirm the supposition that these cells are mobile structures.

The contents of these macrophages are interesting to study, for in their interior are found dead and living leucocytes, nuclei of partially digested leucocytes, together with fragments of such nuclei, micro-organisms healthy and digested, &c. (see further on).

The lymphocytes in the Peyer's patches have been described as small mono- or poly-nucleated cells, with nuclei staining uniformly and deeply with carmine. The nuclei are surrounded by a small amount of protoplasm of a pale rose colour. More careful examination, however, of the lymphoid tissue reveals the presence of other mononucleated lymphocytes, differing only slightly at first from the other cells surrounding them. The nucleus of these cells is somewhat paler and its reticulum slightly more apparent, though the protoplasm may not be at all increased in size as yet (see fig. 1). Other cells possess a nucleus which is again slightly larger, paler, and more vesicular than the latter, as if the nuclear juice had increased at the expense of the solid part of the nucleus (see fig. 2).

It would be very easy to mistake these structures, representing the initial stages of the formation of macrophages, for ordinary leucocytes. The nucleus of others shows a further augmentation, becoming more oval and more like a vesicle in shape (see figs. 3, 4), whilst the protoplasm surrounding it also becomes more abundant (see figs. 5, 6), the whole cell becoming larger. Lastly, the nucleus and protoplasm become enormous (see figs. 7, 8, 8 *bis*), and the macrophage is formed. All the stages in the development of the macrophage may thus be followed in the Peyer's patch. The macrophage is merely a lymphocyte which has assumed a new shape, and, as we shall see, a new function. These cells greatly resemble those found by Heidenhain[1] in the villi of the guinea-pig. True macrophages are not present as a rule in the rabbit's villi, but they are found in great numbers in the Peyer's patches, as well as in the tonsils of many animals.

The macrophages are not scattered in the same numbers throughout the whole Peyer's patch. They certainly do advance right up to the attached border of the epithelial cells, but the superior layers do not contain them in great numbers, few only, two to five, being seen in one field of the microscope.

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[1] Heidenhain, loc. cit.

Occasionally a field may be found in that situation in which they are totally absent. They are not very large as a rule when found near the epithelial lining, though they often already contain leucocytes in their interior, together with microbes in a more or less advanced stage of degeneration. Few microphages are seen in the middle layers of the patches, but the deeper layers are crowded with them. One field of the microscope (Vérick, oc. 3, obj. imm. $\frac{1}{12}$) may contain ten, fifteen, or more of these gigantic cells, crammed almost to bursting with leucocytes and digested micro-organisms. In some places three or more are huddled together, forming a kind of nest, which at first presents an extremely puzzling appearance (see fig. 14). One might almost suppose that they formed a kind of rear-guard, posted in the depths of the lymphoid tissues in order to defend the organism against the attacks of microbes. These cells are, however, but seldom found quite in the lowest depths of the patch, for in most cases there is a layer of five or six lymphocytes between the lowest macrophage and the submucous tissue.

The macrophages found close to the epithelial lining often contain micro-organisms only without any leucocytes (see figs. 8, 8 *bis*), and hence I am inclined to think that, although unable to prove it, the macrophages actually wander to the surface after their prey just as they do in the tonsils. On the other hand, it might be assumed that microphages containing microbes have been swallowed by macrophages, but that the cellulose covering of the bacilli protected them in a more efficient way against the digestive action of the large cells, the microphages being digested far more quickly. Both explanations are probably correct.

It has been stated already that microphages wander as far as the free surface of the intestines, and there seize on micro-organisms. In the superficial layers below the epithelial lining a few of these microphages are always present, the micro-organisms in their interior staining sometimes normally with aniline dyes, sometimes presenting signs of being partially digested. Some microphages are still free, others (intracellular microphages) are found in the interior of macrophages. These cells, after their inclusion in the larger ones, almost always show signs of having been partially digested. In the deeper strata, in which the macrophages are most numerous, the number of intracellular macrophages is often enormous.

It is interesting to know whether the macrophages are able to take healthy leucocytes into their interior or only those already weakened by the struggle with the micro-organisms they contain. Now, if the leucocytes which are wandering about in the patch be carefully examined, after staining with alum-carmine, it will be noticed that the protoplasm of some of them is of a dirty yellow colour, whilst their nuclei are somewhat deformed and contain colouring matters unequally, the whole cell showing an early stage of degeneration. On the other hand, some leucocytes contained in macrophages appear to be perfectly healthy. The fact that I have been able to see similar macrophages containing leucocytes within their interior in the rabbit's mesenteric glands, and also in the spleen, leads me to conclude that macrophages are able to take into their interior and digest apparently healthy leucocytes. Nevertheless it is a fact that many of the intracellular leucocytes are microphages loaded with micro-organisms (see figs. 9, 10, 13, 14, 15); and it is plain that the ptomaines and other poisons secreted by these lower forms of life must exert a deleterious influence on the cells containing them, and so weaken these structures, making them less capable of resisting the attacks of the macrophages. I think, therefore, that although the macrophages are able to take into their interior and destroy leucocytes which are to all appearances still healthy, yet they show a preference for the cells already weakened from some cause or other; for instance, the microphages, the vitality of which has been lowered by the secretions of the microbes contained within their interior.

The intracellular leucocytes undergo the following remarkable changes (alum-carmine and Weigert's method). The nuclei first become oblong, pointed, and irregular in shape (see fig. 10 *a*). At the same time the protoplasm of the cell stains of a characteristic yellowish-grey colour (see figs. 10, *a*, 13, *a*). Occasionally two or more nuclei of the same cell are joined together by a bridge of protoplasm, which stains red, whilst in other cases the nuclei become extremely irregular (see figs. 11, *a*, 13, *a*). In later stages the nuclei lose their power of retaining the colouring matter, part of the nucleus staining deep red, whilst the remainder is rose coloured or yellowish-grey (see figs. 10, *b*, *c*, 11, *b*). At the same time the protoplasm of the cell becomes of a yellowish-grey colour, and looks as if swollen up and increased in size (see fig. 11, *c*, *d*). A later stage is characterised by the fact that the nuclei lose their power of retaining colouring matters at all, and form small greyish-brown masses (see fig. 11, *e*, *f*). Then the nuclei finally disappear, and the leucocytes are represented by greyish round masses which may or may not contain bacilli in their interiors (see figs. 9, *a*; 10, *d*; 11, *g*; 12, *a*, *b*, *c*; 13, *b*; 14, *a*, *b*, *c*, *d*, *e*, *f*, *g*; and 15, *a*, *b*). Many of the degenerated leucocytes are surrounded by a clear space or vacuole (see figs. 10, *b*, 13, *a*). Nuclei ofter appears to offer greater resistance to the digestive power of the macrophages, for partially digested nuclei of leucocytes are found in these large cells, at a time when the remainder of the cell has already disappeared (see fig. 10, *b*, *c*). These fragments of nuclei are often also surrounded by a vacuole (see fig. 10 *b*).

Vacuoles now appear in the intracellular dead leucocytes (see fig. 10, *d*), or else the cell looks as if its peripheral parts were being slowly eaten away (see fig. 14, *h*, *i*). The half-digested cells now become very irregular in shape, whilst the vacuoles increase in size and number. Lastly, the cells become smaller through this process of digestion, more and more irregular at the same time, and the dead leucocytes are finally represented by small masses of dirty-looking yellowish-green protoplasm (see figs. 12, *e*; 13, *c*, *d*, *e*; 14, *j*, *k*, *l*).

It is impossible to say for certain whether the larger macrophages absorb and digest the smaller ones. But although unable to follow the stages of this process, I have often seen in the larger cells débris of nuclei, greatly resembling those of the small macrophages; so that this same inclusion and intracellular digestion is probably true for them also (see figs. 10, *e*; 12, *f*; 13, *f*).

The intracellular destruction and digestion of micro-organisms taking place in the interior of the Peyer's patches remain to be described. Few micro-organisms only are seen in the tissues near the epithelial border, or in the upper and middle layers of the patch. The deep, though not the deepest parts of the patch, contain the largest number of them, and in the rabbit they are found there in almost incredible numbers; 150, 200 or more in one field of the microscope (Vérick, oc. 3, obj. immers. $\frac{1}{12}$). Careful staining reveals the fact that all the micro-organisms are in the interior of the cells, and never lying free between the cells; never in the vessels or in the submucous muscular and peritoneal coats, or even in the deepest layers of the lymphoid tissue. The lymphoid tissue might be compared to a filter, or more aptly to a battle-ground for cells and micro-organisms; the former always proving victorious in healthy animals.

Some of the micro-organisms found within the cells react normally to the colouring matter (see fig. 8, *a*, *b*, *c*) (Weigert's method), but others show the following changes. Some stain of a paler colour, but uniformly (fig. 15, *α*, *β*), whilst others do not retain the colouring matter at all in the centre, though the peripheral parts still react normally staining to reagents (fig. 15, *γ*, *δ*). Others present a mottled appearance (fig. 9, *a*), whilst others, again, resemble strings of micrococci as their different parts take up the gentian-violet unequally. Some bacilli deeply stained at each end show a central part which is quite pale and almost colourless (fig. 10, *a*). Others, again, are irregular in shape (figs. 15, *δ*, 14, *a*). It is quite possible that the mode of decolouration varies according to the kind of micro-organisms, to their vitality, or according to the digestive power of the micro- or macro-phage containing them.

In a more advanced stage of degeneneration the micro-organisms lose their power of absorbing and retaining aniline dyes, but take up alum-carmine or logwood instead. Some of them are then of a pale-red or even deep-red colour (figs. 8, *δ*, 13, *a*). Sometimes part of a bacillus is stained with carmine, the other part taking up gentian-violet, and a bacillus may be thus formed of little points staining red and blue alternately.

In a still more advanced stage the micro-organisms do not retain either carmine or gentian-violet, and appear as little rods or dots, which are extremely pale, and look like the shadows of microbes. The rods then breaking up, finally become granular, irregular in shape, and refract light somewhat more strongly. That is the last stage of the intracellular destruction of micro-organisms.

If the reader will turn to the descriptions that M. Metschnikoff has given of the intracellular destruction of micro-organisms in pathological conditions, he will see how closely the physiological processes as seen in the Peyer's patch resembles the pathological one; how, in fact, both are one and the same process.

A few words may now be said concerning the phagocytic processes in the dog's and guinea-pig's Peyer's patches. Macrophages are always present in these as in the rabbit's lymphoid organs, but they are rather scarce compared with the numbers found in the latter. Micro-organisms undergoing the process of intracellular digestion are found in them also, but in lesser numbers than in the last-named animal. Moreover, it is a peculiar fact that, whereas the microbes in the rabbit's tissues stain readily with Gram's method, this is not the case with those found in the dog's or guinea-pig's cells. To demonstrate their presence in these animals, various reagents have to be used (e.g. Löffler's blue, fuchsine, methyl-violet, &c.). Even then, however, they retain the colouring matter badly, and are always pale, and almost colourless. It is probable, that, if micro-organisms appear to be less numerous in the dog's and guinea-pig's Peyer's patches, this is merely due to our methods for staining intracellular micro-organisms being as yet very imperfect; for, although few healthy microbes appear to be present, a number of degenerated ones were seen, more especially in the guinea-pig's macrophages. In the macrophages of the guinea-pig's small intestine likewise I have also frequently seen micro-organisms. These, however, are difficult to stain, and only assume a pale red colour even when subjected to the action of a strong fuchsine solution.

It has already been stated that many leucocytes force their way through the epithelial layer of the rabbit's tonsils to the free surface of these organs. These mono- and poly-nuclear leucocytes resemble, both in shape and function, those coming to the epithelial border of the rabbit's Peyer's patch. They sometimes contain in their interior small masses of carbon and other extraneous matter which they have evidently fetched from the free surface of the tonsils; for it is impossible to explain, by a mere passive process, the penetration of these dead and indigestible matters into the interior of the cells.

The dog's leucocytes, near the free surface of the tonsils, are often quite full of these masses of carbon and extraneous substances. The greater amount of foreign matter in the leucocytes of the dog's tonsils may, I think, be accounted for in the following manner. In the first place, rabbits breathe through the nose only, whereas dogs breathe through the mouth also. Hence a larger quantity of inhaled dust must pass over the dog's tonsils, remain fixed there, and ultimately be carried away by the animal's leucocytes. In the second place, rabbits feed on clean vegetable food which contains but little dust or foreign matter. Dogs, on the other hand, roaming about the streets, not only poke their noses into most dirty places, but have a habit of taking as food all kinds of refuse. Some of the dust, coal, &c., covering that filthy food, must stick to the mucous surface of the tonsils and become a prey of the ubiquitous leucocytes.

More minute examination of the epithelial layer covering the rabbit's tonsils reveals the presence of other and much larger cells. These have a single clear nucleus staining of a rose colour with alum-carmine, and contain in their interior peculiar and characteristic yellowish-grey round bodies.

These large cells exactly resemble and are indeed macrophages, similar to those found in the Peyer's patches, the intracellular yellowish-grey bodies being simply digested leucocytes. It would be a useless repetition to describe here again the formation of these cells. All that need be said is, that they are developed from the lymphocytes of the tonsils in the same way that the macrophages of the Peyer's patches are developed from the small round-cells of the lymphoid tissues. They never attain quite the same size in the tonsils as in the intestines, but they appear to be more mobile as they are met with close to the free surface. Although present in the lymphoid tissues of the tonsils they are not very numerous there.

The macrophages of the dog do not, as a rule, advance into the epithelial layer, but huge agglomerations of macrophages, forming nest-like masses of cells, are often found just below the epithelial border. These macrophages are crammed with dust, carbon particles, and all kinds of foreign matter (figs. 16, *a*, *b*; 17, *a*, *b*, *c*, *d*; 18, *a*; 19, *a*). They also contain dead leucocytes, together with the extraneous matter which the latter had fetched from the surface (figs. 17, *e*, *f*, *g*; 18 *c*, *d*; 19, *b*). These agglomerations of cells are sometimes so large that, on cutting across a dog's tonsil after soaking it in paraffin, small black dots are seen with the naked eye, which prove to be masses of these large cells. These nests resemble, in all particulars, those found in the rabbit's Peyer's patches.

If the sections be stained with Löffler's blue or gentian-violet, some of the macrophages in the rabbit and dog are found to contain numbers of micro-organisms (see figs. 18 and 19). These are generally micrococci, though bacilli are also found. Some of these micro-organisms present normal staining reactions, but others do not retain aniline dyes and show all the appearances of intracellular digestion which have been described in the Peyer's patches. Although unable to obtain a man's fresh healthy tonsils, I have, in three healthy men and one woman, met with many leucocytes full of microbes on examining the mucus removed from the surface of these lymphoid organs.

III.

The pathology of the tonsils has not attracted sufficient attention as yet, in spite of the fact that clinicians know that many of the local infectious processes in the neck, and some general septic diseases, follow on primary inflammation of the lymphoid organs of the mouth. Although Ludwig[1] in his paper on cellulitis of the neck did not state that this was often secondary to primary disease of the tonsils, Cnopf[2] showed in 1849 that the pathological condition known as angina Ludovici was frequently preceded by inflammation of these lymphoid organs. Von Thaden[3] in 1872 also noticed this sequence of events. Bickel[4] believed that the lymphoid tissue of the throat arrested infectious processes, and he tried to show that to this arrest was due the great number of infectious processes localised in these parts, and in the portions of the pharynx possessing a large amount of lymphoid tissue. Trousseau, using his characteristic descriptive language, said that infectious disease loved the throat.

Though a septic process may begin and end in the tonsils, it is sometimes followed by a general infectious disease. Bergen,[5] as early as 1757, noticed that in some epidemics of tonsillitis inflammation of the testicles came on, after that of the lymphoid organs of the mouth. In 1880, Professor Bouchard[6] stated that he had on four occasions seen tonsillitis followed by infectious nephritis, the urine in two cases containing numerous micro-organisms. In one case the patient fell into a typhoid state and died. Bouchard has also noticed temporary albuminuria during the course of tonsillitis. Mr. Berkeley Hill[7] has recorded a case of angina Ludovici with tumefaction of the left tonsil which was accompanied by albuminuria. Bouchard[1] has described cases followed by pseudo-rheumatism, and Joal[2] has seen ovaritis and orchitis come on after inflammation of the tonsil.

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[1] Ludwig, 'Würt. Corr. Blatt.,' 1836, t. iv, No. 4.

[2] Cnopf, 'Deut. Klinik von A. Joschen,' 1849.

[3] v. Thaden, 'Schmidt's Jahrbücher,' 1872.

[4] Bickel, see Balme, 'Thèse de Paris,' 1888.

[5] Bergen, 'Nova acta phys. nat. curiosorum,' t. i, 1757.

[6] Bouchard, 'Leçons sur les maladies de la nutrition,' 1re édition.

[7] Berkeley Hill, 'Brit. Med. Journ.,' 1881.

Whether the micro-organisms of pneumonia or scarlet fever penetrate or not through the tonsils is an open question, but there can be no doubt that the Bacillus diphtheriæ often attacks the tonsils primarily. The first diphtheritic patch frequently appears on the tonsils, and the researches of MM. Roux and Jersin[3] have proved that diphtheria is a local disease starting and localised in the mucous membrane, the severe general symptoms being produced by the soluble poisons secreted by pathogenic organisms, which are absorbed and then circulate in the blood of the diseased animals.

Occasionally, however, the local diphtheritic process opens the way to other microbes. Thus, Löffler[4] has reported two cases of diphtheria in which, after the patients had died, he found streptococci in the heart, liver, spleen, and kidneys. In one of these the diphtheria was the primary disease, in the other it came on during an attack of scarlet fever.

Heubner and Bardt,[5] G. Crooke,[6] Fraenkel and Freudeberg,[7] have also put similar facts on record. Fraenkel[8] has lately reported two very interesting cases of general infection following disease of the tonsils. In the first of these, a retropharyngeal abscess following disease of the right tonsil sank into the pericardium, the patient dying of purulent pericarditis, a right purulent and a left semi-purulent pleurisy being also found at the post-mortem examination. The second case was one of diphtheria of the larynx and pharynx, and subsequent ulcerous endocarditis.

Now, pathogenic organisms are constantly found in the

[1] Bouchard, loc. cit.

[2] Joal, see Balme, loc. cit.

[3] Roux and Yersin, 'Annal. de l'Institut Pasteur,' 1888.

[4] Löffler, 'Mitthei!. a. d. kais. Gesammt.,' Bd. ii, 1884, p. 451.

[5] Heubner and Bardt, 'Berlin. klin. Wochschr.,' 1884, No. 44.

[6] G. Crooke, 'Fortschr. d. Med.,' 1885, No. 20.

[7] Fraenkel and Freudeberg, see Fraenkel.

[8] Fraenkel, 'Zeitschr. f. klin. Med.,' Bd. xiii, Heft i, p. 14.

mouths of healthy subjects even. Staphylococci have been isolated from the normal saliva by many observers, the micro-organism producing pneumonia also, whilst Löffler[1] has cultivated the Bacillus diphtheriæ from a healthy child's mouth.

These microbes must, of course, pass over the tonsils, but if they try and penetrate into the tissues of that organ they are at once seized upon and destroyed by the micro- and macrophages. But if an animal be at all ailing, it is probable that its cells are unable to exert their phagocytic action and that the micro-organisms multiply in the interior of the tonsils.

This theory is based on clinical and experimental data. That peculiar infectious process termed hospital sore-throat does not attack all people living in hospitals, but only those whose health is deteriorated by overwork, a previous attack of the same disease, or some other reason. Local remedies do but little good, but if the general health be looked after the disease is cut short at once, and the phagocytes of the pharynx and tonsils make short work of the pathogenic microbes.

Dr. Charrin and myself[2] have lately attempted to demonstrate the influence of the nervous system on the production of an infectious disease. We had noticed that if 1 c.c. of a culture of the Bacillus pyocyaneus was injected into the trachea of a healthy rabbit, the animal thus inoculated suffered no evil effects either immediate or remote. After ascertaining that the section of one pneumogastric nerve produced no evil effects we introduced, two days after the nerve had been cut, ½ c.c. of a culture of Bacillus pyocyaneus into the trachea. We found that in two animals out of seven death supervened, and that the five others were very ill on the day following the injection, did not take any food, and remained huddled together in one corner with shaggy coats and difficult respiration, the symptoms disappearing after five to seven days only. Now, M. Tchistowitch[1] has recently shown that when harmless micro-organisms are injected into the trachea, the phagocytes of the lungs take them into their interior, kill and digest them; whilst pathogenic bacilli, such as those of the cholera of fowl, force their way between the cells, pass into the general circulation, and multiplying greatly, finally kill the animal. We may suppose, therefore, that as long as the lungs are in their normal state their phagocytes are able to kill and digest the Bacillus pyocyaneus, but that the same cells are unable to fulfil their task when the nerve is cut.

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[1] Löffler, loc. cit.

[2] Charrin and Armand Ruffer, 'Compt. Rend. Soc. d. Biol.,' March, 1889.

M. Pasteur many years ago showed the influence of cold in the production of an infectious disease.

The struggle between micro-organisms and the animal cells is a physiological process always going on in the tonsils, the microphages and macrophages being victorious in the healthy animal. If these structures are weakened from any cause, e.g. over-fatigue, cold, nervous shock, a local disease follows the multiplication of micro-organisms in the tonsils. An increased emigration of phagocytes may limit the process to the tonsils even then, or the micro-organisms may break through the barrier of these cells, invade the animal body, and the disease become a general one.

The same struggle goes on in the healthy Peyer's patches likewise, and the presence of microbes in the normal structure accounts for the fact that infectious processes in the intestines begin, as a rule, in these lymphoid tissues. Here again some pathogenic organisms are often present, others constantly so, but their mere presence is not sufficient to set up disease. The septic vibrio in the rabbit's intestine does that animal no harm; the bacilli of tubercle and typhoid, which most men ingest at some time or other, prove innocuous as a rule, that is as long as men are healthy. But here, as in the tonsils, over-fatigue, deficient food or oxygen may weaken the animal's resistance, that is, the animal's phagocytes may be unable to do their work of destruction properly, and a local or general infection follow.

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[1] Tchistowitch. At the time of writing M. Tchistowitch had not published his paper. Thanks to his kindness I was able to see his very interesting preparations at the Pasteur Institute in Paris.

The pathology of infectious disorders of the alimentary tract must be re-studied from that point of view. The pathologist must now see how the micro- and macro-phages react when typhoid, tubercle, or other micro-organisms have found their way into the intestinal walls. And he must also study the processes going on in these structures when the walls of the alimentary tract are wounded by pressure from within or from without. These and other problems will receive a satisfactory answer only if pathological observations in the post-mortem room be combined with experimental investigations.

Conclusions.

1. The wandering cells of the lymphoid tissues of the alimentary canal have the power of proceeding to the free surface of such tissues and of taking into their interior lower micro-organisms and foreign matter (charcoal, &c.).

2. There are two kinds of wandering cells in the lymphoid tissues of the alimentary canal :

   a. Microphages (small mono- or poly-nucleated cells).
   b. Macrophages (large mononucleated cells).

3. The macrophages are developed from the small mono nucleated lymphocytes.

4. Macrophages are able to swallow microphages (leucocytes) and to destroy and digest them.

5. The micro-organisms are rapidly destroyed in the interior of the micro- and macro-phages.

6. Micro-organisms are never found lying free between the cells or in the blood-vessels and lymphatics.

7. The destruction of micro-organisms taking place in the normal lymphoid tissues of the alimentary tract resembles in all particulars the destructive process following on the inoculation of pathogenic organisms into resistant animals.

EXPLANATION OF PLATE XXXI,

Illustrating Mr. Armand Ruffer's paper "On the Phagocytes of the Alimentary Canal."

For particulars see text.

Figs. 1—7 represent the stages in the development of a macrophage. (Zeiss, $3 + \frac{1}{18}$.)

Fig. 1. Lymphocyte. The nucleus is somewhat paler and more reticulated than other mononucleated cells. (Zeiss, id.)

Fig. 2. Lymphocyte, with a slightly larger nucleus. (Zeiss, id.)

Fig. 3. The nucleus is increased in size and contains several nucleoli. The amount of protoplasm is also larger. (Zeiss, id.)

Fig. 4. Shows some increase in the amount of protoplasm. (Zeiss, id.)

Fig. 5. Nucleus and protoplasm are both much larger. (Zeiss, id.)

Fig. 6. Smaller macrophage. (Zeiss, id.)

Fig. 7. Macrophage irregular in shape. *a.* Nucleus with well-marked nucleolus (*b*), and intranuclear network. *c.* Protoplasm. (Zeiss, $4 + \frac{1}{18}$.)

Fig. 8.—Macrophage. α, β, γ. Healthy bacilli. δ. Partially digested bacillus. (Zeiss, $4 + \frac{1}{18}$.)

Fig. 8 *bis*.—Macrophage containing bacilli. (Zeiss, id.)

Fig. 9.—Macrophage containing a partially digested macrophage (*a*), which had taken into its interior and digested a bacillus (*a*). (Zeiss, id.)

Fig. 10.—Macrophage. *a, d.* Partially digested leucocytes. *b, c, e.* Fragments of partially digested nuclei. *b, c.* are surrounded by vacuoles. *a.* Partially digested micro-organism. (Zeiss, id.)

Fig. 11.—Macrophage, containing:—*a, b, e, f.* Nuclei in more or less advanced stages of degeneration. *c, d, g.* Partially digested leucocytes. (Zeiss, id.)

Fig. 12.—Macrophage, containing:—*a, b, c, d, e.* Leucocytes in various stages of intracellular destruction. *f.* Nucleus of digested macrophage? (Zeiss, id.)

Fig. 13.—Macrophage, containing:—*a.* Partially digested leucocytes, surrounded by a vacuole. Two healthy bacilli are found in its interior. *b.* Digested microphage with degenerated bacillus (*a*). *c, d, e.* Remnants of digested leucocytes. *f.* Partially digested nucleus (macrophage?). (Zeiss, $3 + \frac{1}{18}$).

Fig. 14.—A group of three macrophages, containing :—*a*, *c*, *g*, *k*. Microphages with more or less digested intracellular bacilli. Notice especially a very irregular bacillus (*a*). *b*, *d*, *e*, *f*, *h*, *i*, *k*, *j*. Leucocytes in various stages of destruction. (Zeiss, id.)

Fig. 15.—Macrophage, containing :—*a*, *b*. Microphages. γ, δ, point to partially digested bacilli. α, β. Bacilli in the early stage of intracellular destruction. *c*. Vacuole. (Zeiss, 4 + $\frac{1}{18}$.)

Fig. 16.—Macrophage, containing :—*a*, *b*, *c*. Particles of foreign matters (coal, dust). (Zeiss, 3 + $\frac{1}{18}$.)

Fig. 17.—Macrophage, containing :—*a*, *b*, *c*, *d*. Particles of foreign matters. *e*, *f*, *g*. Partially digested leucocytes. (Zeiss, id.)

Fig. 18.—Macrophage, containing :—*a*. Small particle of charcoal dust. *c*, *d*. Partially digested leucocytes. (Zeiss, id.)

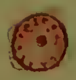
Numerous micrococci are lying scattered throughout the macrophage. *e*. Points to the nucleus. *f*. Points to the vacuole. (Zeiss, id.)

Fig. 19.—Macrophage, containing :—*a*. Particle of charcoal. *b*. Leucocyte, with an included charcoal particle. *c*. Vacuole.

Micrococci in various stages of degeneration are scattered in the cell. (Zeiss, id.)

(N.B.—The macrophages in Figs. 1 to 15 were all drawn from the rabbit's (Peyer's) patch, after staining with alum-carmine, and Gram's method. The Figs. 16 to 19 were drawn from the dog's tonsil, after staining with Löffler's blue. The contours of the macrophages and of their contents were always drawn with the help of the camera lucida.)

Fig. 1.



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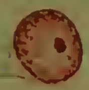

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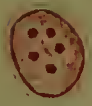

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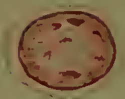

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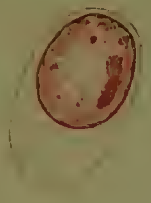

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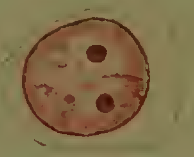

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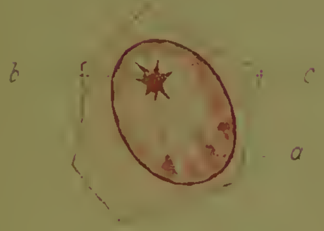

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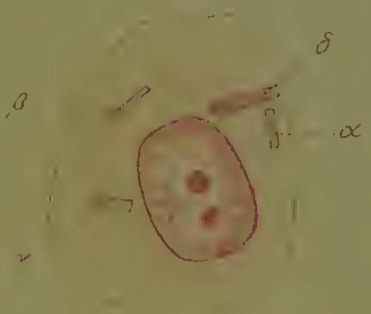
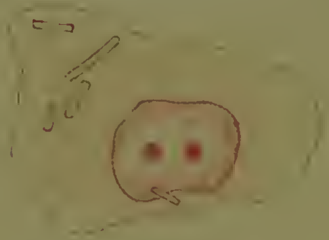


Fig. 8bis



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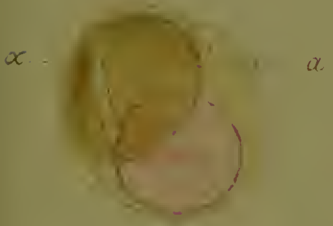

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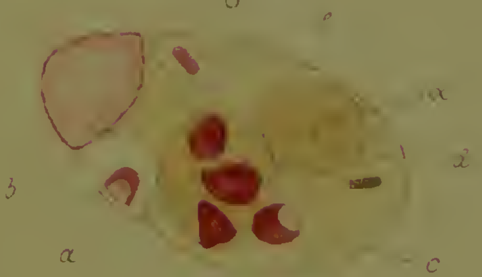

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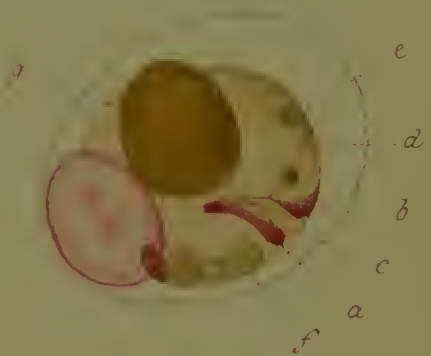

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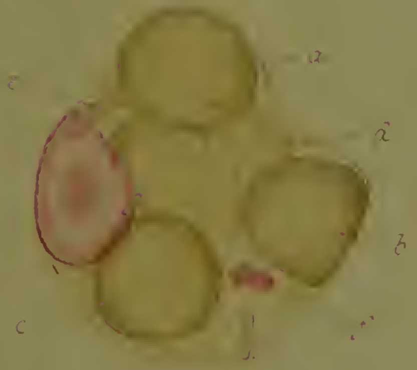

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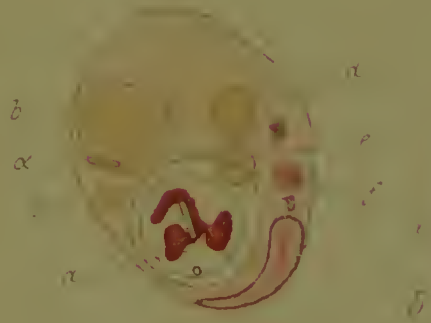

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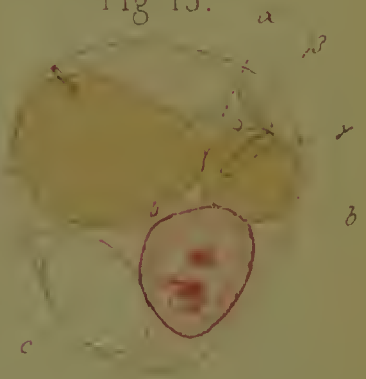

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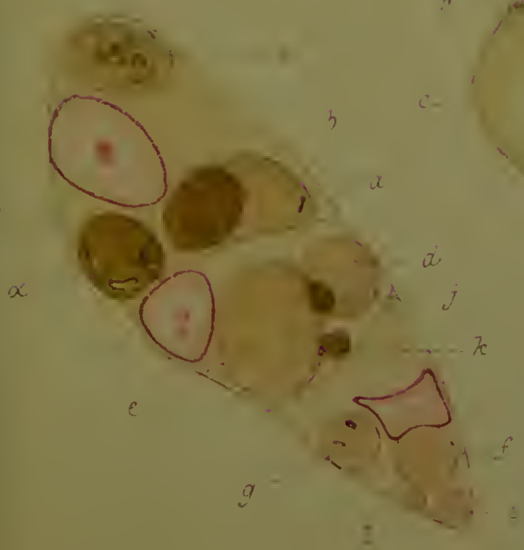

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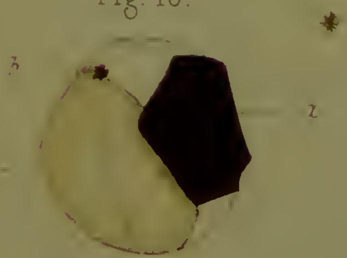

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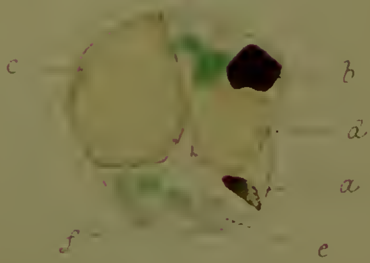

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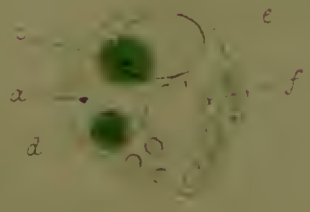

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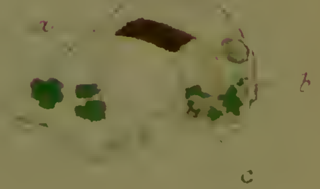

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